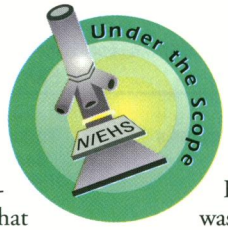
Prime Suspect



Scientists call them the COXs—a pair of closely-related cyclooxygenase enzymes that produce prostaglandins, a class of relatively short-lived hormones that mediate many cellular and physiologic processes. COX-1 is said to be responsible for cellular "housekeeping" functions. COX-2 is necessary for the inflammatory process, but can easily go astray. COX-2 has been observed at the scene—and is the prime suspect—of many a cellular "crime." But the exact role of both enzymes is uncertain. Pharmaceutical companies have developed drugs to contain COX-2 at the first sign of trouble. But scientists at the NIEHS would like to know for sure whether one or both enzymes are guilty of causing damage. And they believe the best way to determine that is by alternately removing each enzyme from experimental animals and observing the effects.

NSAIDs and COXs

Prostaglandins have been the subject of considerable interest in the scientific community because of their role in inflammation. Pain and swelling occur in tissues when cells release prostaglandins in response to physical damage or infection, increasing the permeability of the capillary walls of blood vessels and attracting immune system cells to the damaged tissues. With illnesses such as rheumatoid arthritis, however, joints can become chronically inflamed when prostaglandins are released in the absence of any obvious infection or physical damage.

The recent development of synthetic nonsteroidal antiinflammatory drugs (NSAIDs), such as ibuprofen, represented a breakthrough in the treatment of arthritis and other illnesses involving inflammation. NSAIDs are believed to bring relief by suppressing the COX isoforms that produce pain-inducing prostaglandins. However, the suppression of the COXs also negates the enzymes' beneficial role of protecting the gastric mucosa in the digestive system. The result has been that patients taking high dosages of NSAIDs also suffer a greatly increased incidence of stomach ulcers.

For many years, it was believed that there was only one COX enzyme. However, in 1992, researchers discovered that corticosteroids, which are potent inhibitors of inflammation, blocked the expression of a second enzyme with COX-like activity, subsequently named COX-2. COX-1 is expressed in most tissues and mediates functions including gastric cytoprotection, vascular homeostasis, and normal renal maintenance. COX-2 is normally undetectable in tissues, but can be induced at high levels in macrophages and other cell types in response to proinflammatory stimuli.

The presumed function of COX-2 has sent academic and industry researchers in hot pursuit of a drug that targets only COX-2, while leaving COX-1 to maintain normal homeostatic functions. At stake is a $4 billion business that the pharmaceutical industry has built around the NSAID–COX connection.

COXs and Cancer

While they acknowledge the beneficial effects of NSAIDs, NIEHS researchers want to better understand the biological function of the COXs in disease states. One or both of the enzymes is suspected of playing a key role in colon cancer, skin cancer, certain kidney dysfunctions, and failures in immune cell and reproductive function, among other ailments. The NIEHS has assembled a team of scientists, the Carcinogen Metabolism and Molecular Mechanisms Group, headed by Robert Langenbach, to conduct research aimed at clarifying the functions of the COX enzymes.

Steve McCaw, Image Associates

(left to right) **Pat Chulada, Howard Tiano, Robert Langenbach, Christopher Lee and Burhan Ghanayem.** (Not pictured) **Barbara Davis, Charles Loftin.**

Langenbach's interest in the COXs began in the early 1980s with his belief that the one isoform known at the time might be involved in chemically induced bladder cancers, although he hasn't pursued that particular connection. In 1991, however, Langenbach took a sabbatical at the University of North Carolina at Chapel Hill's Department of Pathology, where he learned a technique that would allow him to pursue research on COXs. At UNC, Langenbach worked with researcher Oliver Smithies, who pioneered the revolutionary technique of "knocking out" specific genes by genetically modifying the DNA of a target gene so that it no longer functions, putting the DNA into a stem cell culture, and inserting the modified stem cells into a developing mice blast. Resulting offspring that carry one copy of the nonfunctioning gene are then bred to produce a strain of "knockout" mice that have no functioning copy of the gene. "The beauty of this technique is that we can have absolute inhibition of a particular gene," says Langenbach. "We can then observe changes in the animals' physiology under normal and stressed conditions, and gain a clearer picture of the gene's function."

Langenbach conducted knockout studies of the COX-1 gene, while Scott Morham, a postdoctoral fellow in the Smithies lab, subsequently took on the disruption of the COX-2 gene. The two groups continue to collaborate and have expanded the effort to include investigators nationwide. At the NIEHS, Langenbach's team is breeding colonies of both COX-1 and COX-2 deficient mice for use in a variety of research projects. Biologist Christopher Lee is responsible for maintaining the proper breeding of animals and using molecular biology techniques to identify the COX-deficient mice to ensure that adequate numbers of specific genotypes are available for all studies.

"The first thing we did was to determine the physiological effects of deleting these genes," says Langenbach. "Preliminary findings showed high incidences of renal failure, altered inflammatory responses, and fertility difficulties in one or both of the knockouts. These discoveries have opened the door to a host of projects looking at the role of the COXs in a variety of diseases."

Already, certain findings have overturned widely held assumptions about the

role of the COXs and NSAIDs. Toxicologist Pat Chulada is leading an effort to define the specific roles of COX-1 and COX-2 in various phases of inflammation. Chulada's approach involves inserting an air pouch beneath the skin of test mice and inflating it over several days, then injecting an irritant into the air pouch to induce an inflammatory response that mimics that of an arthritic joint. "The hypothesis was that COX-2 deficient mice would not be able to mount an inflammatory response," Chulada says. "However, our initial studies show that both COX-1 and COX-2 deficient mice do mount inflammatory responses, although these differ from that of wild-type mice." Chaluda is also examining how the inflammatory process varies when subjected to various carcinogenic and noncarcinogenic stimuli.

Toxicologist Burhan Ghanayem and Chulada also found that, contrary to expectations, COX-1 deficient mice did not show an increase in stomach ulcers. In fact, the COX-1 deficient mice were actually more resistant to NSAID-induced stomach ulcers than the wild type. These results indicate that NSAIDs may reduce inflammation through mechanisms other than or in addition to the suppression of COXs. Further, they bring into question the assumption that a COX-2 suppressing drug would be a more effective antiinflammatory agent with less toxic side effects than current NSAIDs. Critical findings could come when the team compares the health of COX knockouts with NSAID-treated, wild-type mice.

Despite the questions they raise concerning how NSAIDs work, Langenbach sees the research as a win-win situation. "It gives us a model to ask whether NSAIDs have effects in addition to inhibiting the COXs," he says. "Either they work by inhibiting these enzymes, or they work by alternative mechanisms. In either case, it narrows the field in which we have to look for answers."

Chulada is also investigating the role of the COXs in colon cancer development. Recent studies indicate that regular use of NSAIDs not only reduces inflammation, but also decreases the incidence of colon cancer in humans by 40–50%. Scientists have suspected that NSAIDs reduce colon cancer by inhibiting cyclooxygenase and decreasing the production of prostaglandins, but the exact mechanism is unclear. Because NSAIDs decrease the incidence of colon cancer in rodents by as much as 90%, Chulada is cross-breeding COX-1 and COX-2 knockout mice that carry a genetic mutation that predisposes them to multiple intestinal adenomas and monitoring the resulting hybrid mice for onset of cancer and rates of polyp formation in the colon. These mice will also be used to study the genetic and biochemical changes that occur as they develop cancer. "These studies will allow us to determine if, in fact, NSAIDs exert their anticancer effects by inhibiting COX-1 and COX-2 or by altering other biochemical processes," Chulada says. "More importantly, they will help us to identify the mechanisms by which each COX isozyme contributes to colon carcinogenesis."

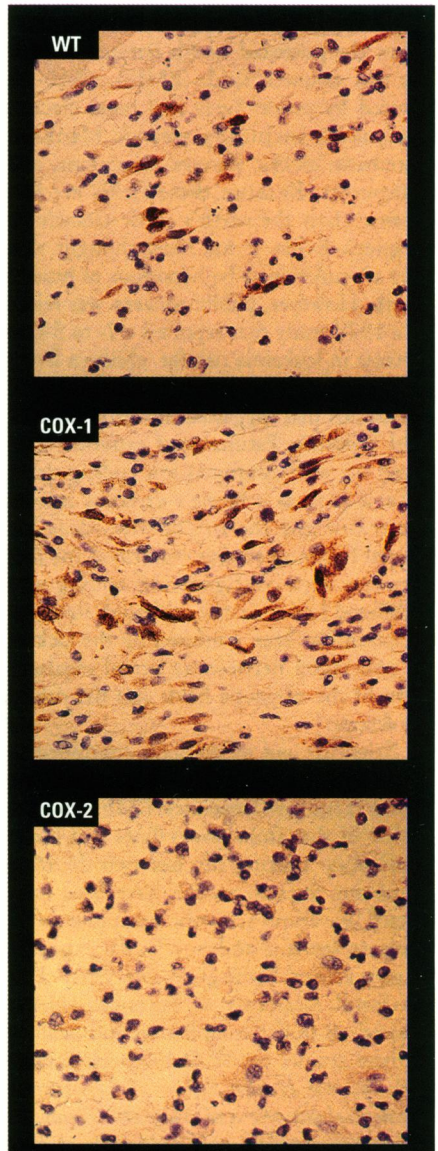
Molecular biologist Howard Tiano is heading a project to examine the effect of COX-1 and COX-2 deficiency in the development of skin cancer in mice. The studies being conducted will allow Tiano and other researchers to study the role of the COXs in both the initiation and promotion stages of skin cancer. In the skin model, as well as Chulada's colon model, NSAIDs are being fed to wild-type and COX-1 and COX-2 deficient mice to determine what biochemical functions other than COX suppression are involved in the carcinogenesis process. "Looking at the role of COXs in two different cancers may give us an indication of their possible involvement in a whole range of cancers," says Langenbach.

Future Developments

Reproductive biologist Barbara Davis is examining how the COXs affect reproductive capabilities. COX-1 is known to have a major role in the onset of labor, and COX-2 is essential for ovulation. Davis is heading a two-pronged study to determine the effect of genetic deletion of COX genes on the reproductive capabilities of mice and the effect of NSAID consumption on these processes. The research has important implications for whether COX-2 specific inhibitors may have unwanted side effects and for whether pregnant women nearing time of delivery should be taking NSAIDs.

In addition to other studies, postdoctoral fellow Charles Loftin is attempting to breed mice that are deficient in both COX-1 and COX-2. Such doubly deficient mice, when compared to COX-1 and COX-2 deficient mice, would provide useful models for determining the necessity of prostaglandins for basic biological processes.

Langenbach anticipates that the NIEHS research effort involving the COXs will continue for several years, evolving in focus and level of understanding as questions are answered and new ones posed. "The COXs are involved in such crucial biological functions that one line of research invariably informs another," he says. "As we uncover the basic biological functions of COXs in normal and stressed states, their role in specific illnesses should become clear. And that should allow scientists to design drugs that more accurately prevent and treat inflammatory diseases."



Selective inhibition. Prostaglandins, thought to be produced by COX-2 during inflammation, are evident (brown pigment) in the macrophages and fibroblasts of air pouch tissue of wild type (WT) and COX-1 knockout mice treated with an inflammatory agent. In tissues of treated COX-2 knockout mice, no protein and minimal prostagladins are produced.

John Manuel